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Our studies on Grant CH-21239 were continued for a final year at Duke University (the previous 3 years were funded at Oklahoma State University). During the past year, our work has developed into two major areas: studies of sodium taurocholate micelles and development of the fluorescence lifetime "filtering" concept. Both of these areas are central to the continued development of fluorescence lifetime selectivity in multicomponent analysis. Our conclusions are that (1) sodium taurocholate and other bile salt micelles should be valuable for the solubilization of fluorescence molecules in aqueous solution, without promoting photophysical interactions between molecules to the extent that they are promoted in detergent micellar solutions; (2) phase-resolution provides more flexibility in fluorescence lifetime filtering, with short-pass and bandpass types of filtering, relative to time-domain (pulsed excitation) techniques, which are restricted to long-pass filtering effects.

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PREFACE

The results described in this section are for work performed in the final year of this project, at Duke University. Previous work on this project, performed at Oklahoma State University, was described in a prior FINAL REPORT.

A. Statement of the Problem Studied

The determination of analytes in real samples is often complicated by several factors, including the presence of multiple components contributing to the observed signal, background signals and matrix effects. As sample complexity increases, selectivity becomes an increasingly important consideration in choosing an appropriate analytical technique. Fluorescence spectroscopy is a highly selective, powerful tool for the analysis of complex samples because of the multiple dimensions of information inherent to the fluorescence phenomena. These dimensions, which are generally independent, include the excitation spectrum, emission spectrum and excited state lifetime. Additional selectivity can be obtained, where appropriate, by (1) other related phenomena such as polarization and quenching, (2) the coupling of fluorescence measurements to separation techniques such as HPLC, and (3) the use of selective reagents such as enzymes or antibodies.

Our research has focussed on the use of fluorescence lifetime techniques to increase the selectivity of fluorescence spectral determinations, with emphasis on multicomponent analysis, suppression of interferences and characterization of complex samples. We have also been exploring bile salt micelles, as an alternative to detergent micelles, for solubilizing fluorescent molecules in aqueous solution. Our goal is to find a reagent that will individually wrap fluorescent molecules, in order to minimize determination errors due to photochemical interactions of fluorescent molecules with each other and with the sample matrix, and to integrate the use of the reagent into phase-resolved fluorimetric determinations of complex samples.

B. Summary of the Most Important Results

1. Fluorescence Lifetime Filtering

Our most fundamental contribution from the past project period has been the comparison of time-resolved and phase-resolved fluorescence techniques in terms of their fluorescence lifetime "filtering" capabilities, in other words, the ability to selectively enhance the intensities of compounds as a function of their fluorescence lifetimes. In time-resolved techniques, selectivity is controlled by a single parameter, time. Filtering is restricted to a "long-pass" effect, i.e., long-lived emission can be selectively enhanced relative to short-lived emission by measuring the intensity after an appropriate delay time. It is not possible, on the other hand, to selectively enhance short-lived signals relative to longer-lived signals, nor to create bandpass-type windows in which emission within a given lifetime range is enhanced relative to both shorter- and longer-lived signals. These types of effects would have

to be achieved indirectly by means of post-acquisition data analysis.

Phase-resolution offers capabilities that complement the time-resolved technique. The two experimental parameters of detector phase angle and modulation frequency can be combined in various ways to achieve either short-pass or bandpass filtering effects. The detector phase angle determines the form of the filter, which includes a short-pass effect, a symmetric bandpass effect, and intermediate, asymmetric bandpass effects. The only effect that cannot be rigorously achieved is the long-pass effect (hence, the complementary nature to time-resolution), although this effect can be approximated in some cases by the use of appropriate bandpass conditions.

Whereas detector phase angle determines the shape of the filtering effect, modulation frequency determines the lifetime range, i.e., the effective width of the filter. Multifrequency phase-modulation instruments allows scanning of a particular filter over a wide range of lifetimes, thereby providing a continuous dimension of fluorescence lifetime information that is readily combined with the dimensions of emission and excitation spectral information in measurements of phase-resolved fluorescence intensities.

2. Multidimensional Data Formats

We have worked with Professor Donald Burdick and his graduate student, Xin-Ming Tu, on the development of data analysis methods for multidimensional data formats that incorporate fluorescence spectral parameters (emission and excitation wavelengths) with fluorescence lifetime parameters (modulation frequency). Routines have been developed for multiway array analysis, and are being applied to multicomponent samples.

3. Studies of Sodium Taurocholate Micelles

Our studies of the bile salt, sodium taurocholate (NaTC) have led to a better understanding of the fundamental aggregation properties of NaTC, including interactions with fluorescent probes both above and below micellar (or "quasi-micellar") concentrations.

C. List of Publications (Comprehensive)

Doctoral Dissertation:
Keith R. Vitense, "Simultaneous Determination of Metals Using Phase-Resolved Fluorescence Spectroscopy", Oklahoma State University, 1988.

Research Publications: See attached list.

D. Participating Scientific Personnel (at Duke)

Dr. Kasem Nithipatikom	Postdoctoral Research Associate
David W. Millican	Graduate Research Assistant

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